ABSTRACT

Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Biochemical Sciences

Candidate: Libuše Černá
Supervisor: PharmDr. Miloslava Netopilová, Ph.D.
Title of diploma thesis: Immunodetection of enzymes catalyzing karbonyl reductions (Western blotting)

The enzymes which reduce carbonyl group are important for metabolism of eobiotics and xenobiotics. There are recognized three superfamilies of carbonyl-reducing enzymes: aldo-keto reductases (AKR), the medium-chain dehydrogenases/reductases (MDR) and the short-chain dehydrogenases/reductases (SDR). The 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is member of the SDR superfamily. This enzyme is essential for activating steroid hormone cortison to its active form cortisol. Its activity is also vital for metabolism of some other xenobiotics, e.g the anticancer drug oracin. Yet the carbonyl group of oracin is also reduced by another unknown enzyme wich is located in microsomal membrane. For the purification of this unknown enzyme Q Sepharose was used. The aim was to find out which fractions after purification contain 11β-HSD1 by using the imudetection (Western blotting). In Western blotting we used nitrocelulose membrane, the primary rabbit polyclonal antibodies against 11β-HSD1 dilution 1:1000, the secondary polyclonal swine anti-rabbit immunoglobulins/HRP antibodies dilution 1:1000 and the ECL Western Blotting Detection System (Amersham company). Blotting time for this method was 60 minutes. The incubation time was 60 minutes with the primary antibodies and 90 minutes with the secondary antibodies (both at laboratory temperature). The 11β-HSD1 was found in fractions A11, A12, B12, B11 and B10 which were separated from liver microsomes by Q Sepharose. These fractions are supposed to contain the unknown enzyme. The enzyme 11β-HSD1 was not found in the other fractions.